

WHAT IS CLAIMED IS:

1. A method for testing whether a substance is or contains a cell growth inhibitor that acts by selectively inhibiting the function of a gene product required for cellular growth or survival, wherein the method comprises:
- 5 (A) providing recombinant cells that are capable of expressing an RNA fragment that interferes with the expression of the gene product;
- (B) growing the recombinant cells in a nutrient medium in the presence of the test substance and under conditions which result in (i) the expression of the RNA fragment and down regulation of the synthesis of the gene product and (ii) the loss by the cells of the capability to express the RNA fragment; and
- 10 (C) analyzing the resulting cell growth; wherein:
- (1) if there is essentially no cell growth due to the death of all or substantially all of the cells, then the test substance is or contains a growth inhibitor that does not selectively inhibit the targeted gene product;
- 15 (2) if there is essentially no inhibition of cell growth due to the survival and growth of all or substantially all of the cells, then the test substance is not or does not contain a growth inhibitor; or
- 20 (3) if there is growth inhibition due to the death of a substantial portion of the cells accompanied by the survival and growth of revertant cells having no capability to express the RNA fragment, then the test substance is or contains a growth inhibitor that selectively inhibits the targeted gene product.
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2. The method according to claim 1, wherein the RNA fragment expressed by the cells in Step A comprises an antisense RNA.
3. The method according to claim 2, wherein the antisense RNA is encoded in a plasmid contained in the recombinant cells.
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4. The method according to claim 2, wherein the antisense RNA is encoded in DNA in the genome of the recombinant cells.

5. The method according to claim 1, wherein the recombinant cells are selected from the group consisting of bacterial strains and fungal strains.

5 6. The method according to claim 5, wherein the cells are a strain of bacteria and the substance is being tested to determine whether it is or contains an antibacterial agent that acts by selectively inhibiting the function of a gene product required for the growth or survival of the bacteria.

10 7. The method according to claim 6, wherein the RNA fragment capable of being expressed by the bacteria in Step A comprises an antisense RNA encoded in a plasmid contained in the bacteria.

8. The method according to claim 1, wherein the nutrient medium in which the recombinant cells are grown in Step B is a semi-solid medium inoculated with the test substance; and analyzing the resulting cell growth in Step C; wherein:

- 15 (1) if the semi-solid medium exhibits a clear zone indicative of essentially no growth due to the death of all or substantially all of the cells, then the test substance is or contains a growth inhibitor that does not selectively inhibit the targeted gene product;
- 20 (2) if the semi-solid medium does not exhibit a zone of no growth, due to the survival and growth of all or substantially all of the cells, then the test substance is not or does not contain a growth inhibitor; or
- 25 (3) if the semi-solid medium exhibits a zone of no growth except for one or more small cell colonies in the zone due to the survival and growth of revertant cells having no capability to express the RNA fragment, then the test substance is or contains a growth inhibitor that selectively inhibits the targeted gene product.

9. The method according to claim 8, wherein the recombinant cells are a strain of bacteria and the test substance is being analyzed to determine whether it is or contains an antibacterial agent that acts by selectively inhibiting the function of a gene product required for the growth or survival of the bacteria.

10. The method according to claim 8, wherein the semi-solid medium is an agar plate or an agarose plate.

11. The method according to claim 1, wherein:
the RNA fragment expressed by the cells in Step A comprises an
antisense RNA encoded in a first plasmid, said first plasmid also encoding a repressor
gene that regulates a reporter gene integrated into the genome of the cells or contained
5 in a second plasmid;
the nutrient medium in Step B is a liquid medium;
the loss by the cells of the capability to express the RNA fragment in
Step B is accompanied by the loss of the capability of the repressor to regulate the
10 reporter gene; and
in the analysis of cell growth in Step C:
(1) if there is essentially no cell growth due to the death of
all or substantially all of the cells, then the test substance is or contains
a growth inhibitor that does not selectively inhibit the targeted gene
15 product;
(2) if there is essentially no inhibition of cell growth due to
the survival and growth of all or substantially all of the cells, said cells
having no expression of the reporter gene, then the test substance is not
or does not contain a growth inhibitor; or
20 (3) if there is growth inhibition due to the death of a
substantial portion of the cells in the liquid medium accompanied by
the survival and growth of revertant cells having no capability to
express the RNA fragment but having expression of the reporter gene,
then the test substance is or contains a growth inhibitor that selectively
25 inhibits the targeted gene product.

12. The method according to claim 11, wherein the reporter gene is
encoded in a second plasmid and encodes a fluorescent protein; and the survival and
growth of revertant cells is indicated by the detection of fluorescence from the
30 fluorescent protein.

13. The method according to claim 12, wherein the cells are a
strain of bacteria; and the antisense RNA is a xylose-inducible antisense RNA
encoded in the first plasmid and is capable of interfering with the expression of a gene

selected from the group consisting of fabF, pheT, murA, secA, dnaC, ileS, ftsZ, secI, polC, dnaE, gyrE, gyrA, murB, rpl, parE, and parC.

14. The method according to claim 12, wherein the analysis of cell growth in Step C comprises measuring the level of fluorescence relative to the number of cells, and the number of cells is determined by measuring absorbance.

15. A method for testing whether a substance is or contains an antibacterial agent that acts by selectively inhibiting the function of a gene product required for the growth or survival of a strain of bacteria, wherein the method comprises:

(A) providing recombinant cells of the bacterial strain that are capable of expressing an RNA fragment that interferes with the expression of the gene product;

(B) growing the recombinant cells in a nutrient medium in the presence of the test substance and under conditions which result in (i) the expression of the RNA fragment and the down regulation of synthesis of the gene product and (ii) the loss by the cells of the capability to express the RNA fragment; and

(C) analyzing the resulting cell growth; wherein:

(1) if there is essentially no cell growth due to the death of all or substantially all of the cells, then the test substance is or contains an antibacterial agent that does not selectively inhibit the targeted gene product;

(2) if there is essentially no inhibition of cell growth due to the survival and growth of all or substantially all of the cells, then the test substance is not or does not contain an antibacterial agent; or

(3) if there is growth inhibition due to the death of a substantial portion of the cells accompanied by the survival and growth of revertant bacterial cells having no capability to express the RNA fragment, then the test substance is or contains an antibacterial agent that selectively inhibits the targeted gene product.

16. The method according to claim 15, wherein the RNA fragment expressed by the cells in Step A comprises an antisense RNA.

17. The method according to claim 16, wherein the antisense RNA is encoded in a plasmid contained in the cells.

18. The method according to claim 16, wherein the antisense RNA
5 is encoded in DNA in the genome of the cells.

19. The method according to claim 15, wherein the bacterial strain is selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Haemophilus*, *Enterobacter*, *Acinetobacter*, *Bacillus*,
10 *Stenotrophomonas*, *Burkholderia*, *Salmonella*, and *Pseudomonas*.

20. The method according to claim 19, wherein the bacterial strain is selected from the group consisting of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Bacillus subtilis*, *Stenotrophomonas maltophilia*, *Salmonella typhimurium*, and *Burkholderia cepacia*.
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21. The method according to claim 20, wherein the bacterial strain
20 is a *Staphylococcus aureus* containing a plasmid expressing a xylose-inducible anti-sense RNA for a gene required for the survival or growth of *Staphylococcus aureus*.

22. The method according to claim 21, wherein the xylose-inducible anti-sense RNA expressed by the plasmid is for a gene selected from the
25 group consisting of *fabF*, *pheT*, *murA*, *secA*, *dnaC*, *ileS*, *ftsZ*, *secI*, *polC*, *dnaE*, *gyrE*, *gyrA*, *murB*, *rpl*, *parE*, and *parC*.

23. The method according to claim 15, wherein the target gene of the RNA fragment is encoded to express a fatty acid synthase, an aminoacyl-tRNA
30 synthetase, a protein secretase, a peptidyl transferase, a transglycosylase, a transpeptidase, or a ribosomal associated protein.

24. The method according to claim 15, wherein the nutrient medium in which the recombinant cells are grown in Step B is a semi-solid medium

inoculated with the test substance; and analyzing the resulting cell growth in Step C; wherein:

- 5 (1) if the semi-solid medium exhibits a clear zone indicative of essentially no growth due to the death of all or substantially all of the cells, then the test substance is or contains an antibacterial agent that does not selectively inhibit the targeted gene product;
- (2) if the semi-solid medium does not exhibit a zone of no growth, due to the survival and growth of all or substantially all of the cells, then the test substance is not or does not contain an antibacterial agent; or
- 10 (3) if the semi-solid medium exhibits a zone of no growth except for one or more small cell colonies in the zone due to the survival and growth of revertant cells having no capability to express the RNA fragment, then the test substance is or contains an antibacterial agent that selectively inhibits the targeted gene product.

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25. The method according to claim 24, wherein the semi-solid medium is an agar plate or an agarose plate.

20 26. A method for testing whether a substance is or contains an antibacterial agent that acts by selectively inhibiting the function of a gene product required for the growth or survival of a strain of bacteria, wherein the method comprises:

 (A) providing recombinant cells of the bacterial strain containing a plasmid that is capable of expressing an antisense RNA that interferes with the expression of the gene product; and

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 (B) growing the recombinant cells on an agar plate in the presence of the test substance and under conditions which result in (i) the expression of the antisense RNA and the down regulation of synthesis of the gene product and (ii) the loss by the cells of the capability to express the antisense RNA; and

30 (C) analyzing the resulting cell growth; wherein:

- (1) if the agar plate exhibits a clear zone indicative of essentially no growth due to the death of all or substantially all of the cells, then the test substance is or contains an antibacterial agent that does not selectively inhibit the targeted gene product;

(2) if the agar plate does not exhibit a zone of no growth, due to the survival and growth of all or substantially all of the cells, then the test substance is not or does not contain an antibacterial agent; or

5 (3) if the agar plate exhibits a zone of no growth except for one or more small cell colonies in the zone due to the survival and growth of revertant cells having no capability to express the RNA fragment, then the test substance is or contains an antibacterial agent that selectively inhibits the targeted gene product.

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27. The method according to claim 26, wherein the plasmid is a plasmid that is induced to express the antisense RNA and that is encoded for resistance to an antibiotic.

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28. The method according to claim 27, wherein in Step B (i) the expression of the antisense RNA and the down regulation of the gene product is obtained by growing the cells in the presence of an effective amount of an antisense RNA-inducer and (ii) the loss by the cells of the capability to express the antisense RNA is obtained by growing the cells in the absence of the antibiotic to which the
20 plasmid is resistant.

29. The method according to claim 28, wherein the antisense RNA is a xylose-inducible antisense RNA capable of interfering with the expression of a gene expressing a fatty acid synthase, an aminoacyl-tRNA synthetase, a protein
25 secretase, a peptidyl transferase, a transglycosylase, a transpeptidase, or a ribosomal associated protein.

30. The method according to claim 29, wherein the antisense RNA is a xylose-inducible antisense RNA capable of interfering with the expression of a
30 gene selected from the group consisting of fabF, pheT, murA, secA, dnaC, ileS, ftsZ, secI, polC, dnaE, gyrE, gyrA, murB, rpl, parE, and parC.

31. The method according to claim 30, wherein the bacteria is *Staphylococcus aureus*.